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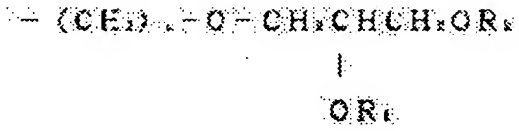
(71)Applicant : WAKO PURE CHEM IND LTD
(72)Inventor : IWATA TSUTOMU
TAKIUCHI KUNIO
FUKUMOTO MASAMI

(54) NEW MODIFIED SILICA GEL

(57)Abstract:
PURPOSE: To provide a packing material for column chromatography easy for production, excellent in separation ability, reproducibility and durability to be used for the analysis of various components in a vital sample (blood serum, urine or the like) and to provide the analytical method of the components in the vital sample by the use of the packing material.
CONSTITUTION: A modified silica gel is made by substituting a part or all of hydrogen atoms in silanol groups on the surface of a silica gel for a group expressed by the formula through silicon atom (in the formula, (n) is integer of ≥3, one of R1 and R2 expresses hydrogen atom and the other expresses −(CH2)mOH (in the formula (m) expresses integer of ≥2) or is made by replacing one part of hydrogen atoms of the silanol groups for the group expressed by the formula and the remaining one or all part by a (substituted) alkyl group and the packing material for column chromatography contains the modified silica gel.



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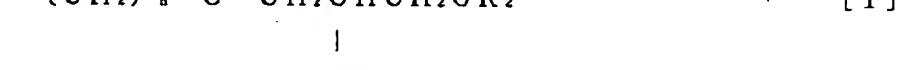
* NOTICES *

CLAIMS

[Claim(s)]
[Claim 1] Surface a part or surface all of a silanol group of a hydrogen atom minds a silicon atom, and it is a general formula [1]. [of silica gel]
[Formula 1]

[1]

[-- n expresses three or more integers among a formula, either of R1 and R2 is a hydrogen atom, and another side expresses -(CH2) mOH (m expresses two or more integers among a formula.).] Denaturation silica gel which came out and was replaced by the radical shown.
[Claim 2] A part of hydrogen atom of a silanol group of the surface of silica gel minds a silicon atom, and it is a general formula [1].
[Formula 2]



[1]

[-- n expresses three or more integers among a formula, either of R1 and R2 is a hydrogen atom, and another side expresses -(CH2) mOH (m expresses two or more integers among a formula.).] Denaturation silica gel which came out, was replaced by the radical shown and was replaced by the alkyl group in which a remaining part or remaining all may have the substituent.
[Claim 3] A bulking agent for chromatographies which comes to contain denaturation silica gel according to claim 1 or 2.
[Claim 4] Analytical method of a component in a biological material using a bulking agent for chromatographies according to claim 3.
[Claim 5] A pretreatment method of a biological material for component analysis using a bulking agent for chromatographies according to claim 3.
[Claim 6] A column for chromatographies which comes to fill up a bulking agent for chromatographies according to claim 3.
[0001]

[Translation done.]

* NOTICES *

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[Industrial Application] This invention relates to the analytical method of the various components in the biological materials (a blood serum, urine, etc.) which used this for the bulking agent for chromatographies which comes to contain denaturation silica gel useful as the bulking agent for chromatographies, especially a bulking agent for high performance chromatography (HPLC), and this denaturation silica gel, and the list.
[0002]
[Background of the Invention] HPLC is widely used as an analysis means of the various components in a biological material (a drug, metabolite, etc.). However, in carrying out the quantum of a drug, metabolite, etc. in the biological material which contains protein components, such as a blood serum, so much using HPLC, in order to remove the evil by adsorption to proteinic column packing material etc., pretreatment of deproteination processing etc. was needed conventionally.
[0003] However, such pretreatment actuation requires great time amount and a great effort, and causes badness of an analytical error and repeatability. Then, the bulking agent for chromatographies for analyzing a direct biological material is developed variously, without performing deproteination processing. However, there can be merits and demerits neither of the bulking agents, and can satisfy them.
[0004] Some of those examples are shown below. As a forerunner of a bulking agent who has such a property, it is protein given in H.Yoshida, I.Morita and G.Tamai, Chem.Pharm.Bull., 30, 3827 (1982) and H.Yoshida, I.Morita, G.Tamai, T.Masujima, T.Tsuru, N.Takai and H.Imai, Chromatographia, and 19,466(1984). Coat There is an octadecyl silyl (protein-coated ODS) bulking agent. It is shown by by this bulking agent's carrying out the coat of the silica surface which has an octadecyl silyl radical (ODS radical) with the denatured plasma protein, and using this bulking agent that analysis of the drug by direct impregnation of a blood serum sample is possible. However, in these bulking agents, if use continues for a long period of time, it has the trouble in the field of separability -- the trouble in the field of endurance or the column of high separation efficiency of denaturation plasma protein eluting which were made to adsorb are not obtained.
[0005] The bulking agent which introduced the hydrophobic radical into the internal surface of porous support, and introduced the hydrophilic radical into the outside surface for the purpose of solving these troubles, and the so-called inside opposition (internal-surface reversed phase) bulking agent were developed. If this bulking agent is used, the protein in the blood serum which is a macromolecule (albumin and globulin) will bypass a column, without not entering in the pore of a bulking agent and adsorbing the outside surface of hydrophilicity, and molecules, such as a moreover comparatively small drug, will become possible [making a hydrophobic internal surface adsorb and dissociating]. There are some which are called the Pinkerton column carried by I.H.Hagestam, T.C.Pinkerton, Anal.Chem., 57, and 1757(1985)., JP,61-65159,A, etc., for example as a bulking agent of such inside opposition. This is obtained by making the carboxypeptidase A which is proteolytic enzyme act, and cutting the phenyl alanyl side chain of an outside surface, after combining oligopeptides (glycyl-phenyl alanyl-phenylalanine etc.) with this through

carbonyldiimidazole by using as a raw material porous silica gel which introduced the glyceryl propyl silyl radical. That is, the bulking agent which has the glycyl-phenyl alanyl-phenylalanine which is hydrophobic ligand in the internal surface of a bulking agent, and has at it the glycyl-glyceryl propyl silyl radical which is hydrophilic ligand in an outside surface is it. However, since this bulking agent has low hydrophobicity, it is unsuitable for separation of a hydrophilic drug or metabolite. Moreover, the trouble that the pH range of a mobile phase usable for dissociation of the carboxyl group of the glycyl residue of an outside surface is limited with pH 6-7.5, and since the enzyme reaction is further used for manufacture, a production process is complicated and it also has troubles, like quality tends to vary.

[0006] Moreover, the thing of a publication is in JP,1-123145,A as a bulking agent manufactured by the similar principle. This is a bulking agent obtained by introducing a hydrophobic radical through amide association under triethylamine existence by making octanoyl chloride react by using as a start raw material the porous silica which introduced the aminopropyl silyl radical, next hydrolyzing the acyl group of an outside surface by polymyxin acylase, making an outside surface generate the amino group, making this react with glycidol, and making an outside surface into hydrophilicity. However, since this bulking agent also uses the enzyme reaction for manufacture, a production process is complicated and it has the trouble that quality tends to vary.

[0007] Furthermore, there is a thing given in JP,5-203636,A as a bulking agent manufactured by the similar principle again. This uses as a raw material porous silica gel which introduced the glyceryl propyl silyl radical, and it is made to react with a fatty-acid chloride under triethylamine existence, and after introducing the hydrophobic radical which consists of fatty acid ester, it is obtained by the method of hydrolyzing the acyl group of an outside surface by lipase (bulking agent which hydrophobic fatty acid ester remains in an internal surface, and has the glyceryl propyl silyl radical which is a hydrophilic group in an outside surface). However, since this bulking agent also uses the enzyme reaction for manufacture, a production process is complicated and it has the trouble that quality tends to vary.

[0008] In order to solve such a trouble, to use the plasma instead of an enzyme and to manufacture a bulking agent is also tried (JP,4-68244,B, JP,4-61809,B, etc.). That is, the method of plasma treatment of the porous silica which introduced hydrophobic radicals (ODS radical etc.), for example being carried out, carrying out desorption of the ODS radical of an outside surface alternatively, then introducing gamma-glycidoxy propyl silyl radical into an outside surface, carrying out ring breakage of the epoxy ring with an acid, considering as a glyceryl propyl silyl radical, and manufacturing the bulking agent of the inside opposition which introduced the hydrophobic ODS radical to a silica outside surface at the glyceryl propyl silyl radical of hydrophilicity and an internal surface is it. However, like [such a manufacture method] the manufacture method using the aforementioned enzyme, a production process is complicated and it still has the trouble that quality tends to vary.

[0009] Moreover, the method of using an acid instead of the plasma and manufacturing a bulking agent is also tried (JP,2-59415,A etc.). That is, it is the method of carrying out desorption of the ODS radical of an outside surface alternatively, then using gamma-glycidoxypropyltrimetoxysilane, and introducing the glyceryl propyl silyl radical of hydrophilicity into a silica outside surface, by carrying out acid treatment of the porous silica which introduced the ODS radical. Although it has the advantage that this process is simple, it has the trouble that the quality of a bulking agent still tends to vary.

[0010] Moreover, manufacture of the inside opposition bulking agent using the reactant difference of a sililation reagent is also tried (JP,64-16962,A etc.). Namely, it compares with the diffusion rate into silica pore. Only a pore outside surface is alternatively silanized [next] by using the sililation reagent (for example, perfluoro-butyl ethylene dimethylsilyl-N-methyl aceto imide which has N-methyl aceto imide radical as a leaving group and which was very much rich in reactivity) whose reaction rate is size. It is the method of manufacturing the bulking agent which has the perfluoro-butyl ethylene dimethylsilyl radical of hydrophilicity in an outside surface, and has an ODS radical in an internal surface, by embellishing an internal surface with octadecyl dimethylsilyl chloride. However, there is still a trouble that the quality of the bulking agent obtained also about this process tends to vary.

[0011] There is also a thing using the reactant difference of the internal surface of porous support and an outside surface given [as the manufacture method of an inside opposition bulking agent] in JP,3-107759,A. gamma-glycide oxy-propyltrimethoxysilane is made to react to a porous silica on conditions [****] (non-catalyst) first, and it embellishes only to an outside surface substantially. Namely, subsequently By the strong reaction condition under N and N-diisopropyl ethylamine (condensation catalyst) existence The method of introducing phenyl trimethoxysilane into an internal surface, processing this from an acid, carrying out ring breakage of the epoxy ring further, and manufacturing the bulking agent of the inside opposition which introduced the hydrophobic phenyl group to an outside surface at the glyceryl propyl silyl radical of hydrophilicity and an internal surface is it. However, also in this method, since there are few reactant differences in the inside-and-outside surface of pore, reaction conditioning is difficult and the trouble of being easy to produce dispersion is in the quality of the bulking agent obtained.

[0012] As stated above, since an inside opposition bulking agent introduced a hydrophilic radical and a hydrophobic radical into an internal surface and an outside surface separately and was manufactured, even if the quality of the bulking agent obtained used these bulking agents as a result of [its] dispersion, it had the trouble [data / with sufficient repeatability] of being difficult to get.

[0013] Then, the hybrid functional phase (mixed functional phase) bulking agent with which a hydrophilic radical phase and a hydrophobic radical phase exist in the internal surface and outside surface of porous support was developed. In this kind of bulking agent, a huge form molecule like protein will be bypassed without adsorbing a hydrophilic radical, and separation adsorption of the comparatively small molecules, such as a drug, will be carried out at a hydrophobic radical.

[0014] There is a thing given in JP,4-40366,A as such a hybrid functional phase bulking agent. This introduces gamma-glycidoxy propyl silyl radical of hydrophilicity into the internal surface and outside surface of a porous silica, next introduces hydrophobic alkyl groups (butyl, octyl radical, etc.) and a hydrophobic phenyl group. And after hydrolyzing a glycidoxy radical and making a diol radical generate, gamma-glycidoxy propyl silyl radical is introduced again, making a glycidoxy radical understand by acid adding water. However, also in this method, although the trouble that it is difficult to prepare the amount of installation of a hydrophobic group and a hydrophilic group improves considerably, the trouble that the separability of the bulking agent obtained etc. is not good remains.

[0015] Moreover, there is also a thing given [as a hybrid functional phase bulking agent with which the hydrophilic radical and the hydrophobic radical were introduced into the porous support covered with silicon polymer] in JP,5-72190,A. This covers porous support with the polymer which has a SiH radical, makes the hydrophobic radicals (styrene, 1-octene, 1-octadecene, etc.) which have a double bond, and a hydrophilic radical (polyol) react to the SiH radical of this polymer, and is manufactured. However, there are troubles, like separation of the alkali of the bulking agent obtained since it is difficult that the actuation at the time of manufacture is complicated, that it is difficult to prepare the amount of installation of a hydrophilic radical and a hydrophobic radical, and to gather the coverage in the polymer which has the SiH radical of porous support etc. is not good also in this method.

[0016] There is a bulking agent with which calling it a Binary-Layered Phase bulking agent with the bulking agent embellished with the alkyl silyl radical having two different functions of the function as a hydrophilic radical and the function as a hydrophobic radical for a porous simple substance on the other hand, i.e., Nimura etc., is proposed [collection [of the 36th liquid chromatography study group lecture summaries] p.68(1993).]. A hydrophilic portion is not adsorbed, but this kind of bulking agent bypasses macromolecules, such as protein, and comparatively small molecules, such as a drug, have the function to adsorb and separate into a hydrophobic portion. As such a bulking agent, there is a bulking agent given [for example,] in JP,64-16961,A. That is, the bulking agent which introduced the functional group which embellished the diol of glyceryl propyl dimethylsilane in the ketal mold has been obtained using the sililation reagent which has N-methyl acetamido radical etc. as a Lee Byng atomic group in

porous support. However, the engine performance of this bulking agent was inferior to the bulking agent (2 area opposition bulking agent) with which a hydrophilic radical and a hydrophobic radical given in JP,64-16962,A were separately introduced as indicated in the above-mentioned open official report.

[0017] Moreover, there is a bulking agent given [as a bulking agent with which the portion which introduces the radical having two different functions from the function as a hydrophilic radical and the function as a hydrophobic radical into the internal surface and outside surface of a porous silica, and has a function as the hydrophilic radical has a form of diol] in JP,2-45758,A. This is 5 and the bulking agent embellished with 6-dihydroxy hexyl silyl radical obtained by carrying out acid treatment, after introducing 5 and 6-epoxy hexyl silyl radical etc. into silica gel. Moreover, after compounding what changed into butyl, methylbutyl, and hexyl the propyl portion of the gamma-glycidoxypropyltrimetoxysilane currently used from the former and introducing gamma-glycidoxyalkyl group into a porous silica by Y.Sudo and others using this, the bulking agent obtained by carrying out ring breakage of the epoxy ring from an acid is also reported (17 Y. Sudo, M.Akiba, T.Sakaki and Y.Takahata, J.Liq.Chromatogr. 1743 (1994)). Although there is an advantage that these bulking agents are simple for adjustment of the amount of installation of a hydrophobic radical and a hydrophilic radical, it has separation of the peak of a drug, or the trouble of not being good, in analysis of a hydrophilic drug.

[0018]

[Objects of the Invention] This invention was made in view of the above-mentioned **** condition, and is easy to manufacture. Moreover, separability, The denaturation silica gel [excellent in repeatability and endurance] useful as a bulking agent for column chromatography used for analysis of the various components in biological materials (a blood serum, urine, etc.), It aims at offering the column for chromatographies which comes to contain this bulking agent in the analytical method list of the component in the biological material using the bulking agent for chromatographies and this which come to contain this denaturation silica gel.

[0019]

[Elements of the Invention] Surface a part or surface all of a silanol group of a hydrogen atom minds a silicon atom, and this invention is a general formula [1]. [of (1) silica gel]

[0020]

[Formula 3]

$$-(CH_2)_n-O-CH_2CHCH_2OR_2 \qquad [1]$$
$$\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\quad OR_1$$

[0021] [-- n expresses three or more integers among a formula, either of R1 and R2 is a hydrogen atom, and another side expresses -(CH2) mOH (m expresses two or more integers among a formula.).] It is invention of the denaturation silica gel which came out and was replaced by the radical shown.

[0022] Moreover, a part of hydrogen atom of the silanol group of the surface of silica gel minds a silicon atom, and (2) this inventions are general formulas [1].

[0023]

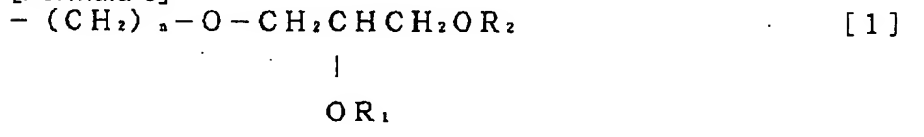
[Formula 4]

$$-(CH_2)_n-O-CH_2CHCH_2OR_2 \qquad [1]$$
$$\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\quad OR_1$$

[0024] [-- n expresses three or more integers among a formula, either of R1 and R2 is a hydrogen atom, and another side expresses -(CH2) mOH (m expresses two or more integers among a formula.).] It is invention of the denaturation silica gel which came out, was replaced by the radical shown and was replaced by the alkyl group in which a remaining part or remaining all may have the substituent.

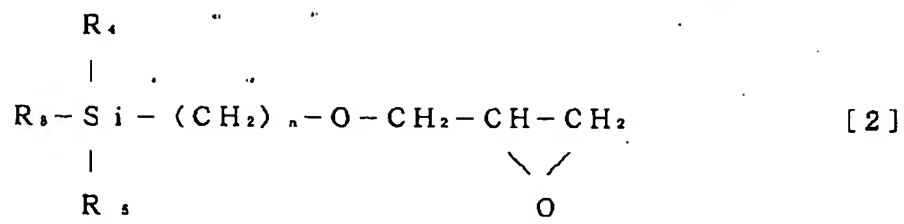
[0025] Furthermore, this invention is invention of the bulking agent for chromatographies which

comes to contain the denaturation silica gel of a publication in (3) above (1) or (2).
[0026] Furthermore, this invention is invention of the analytical method of the component in the biological material which uses the bulking agent for chromatographies of a publication for (4) above (3) again.
[0027] Moreover, this invention is invention of the pretreatment method of the biological material for component analysis which uses the bulking agent for chromatographies of a publication for (5) above (3).
[0028] Furthermore, this invention is invention of the column for chromatographies which comes to fill up (6) above (3) the bulking agent for chromatographies of a publication.
[0029] Namely, as a result of repeating research wholeheartedly that this invention persons should attain the above-mentioned purpose, a silicon atom is minded for surface a part or surface (the internal surface and outside surface) all of a silanol group of a hydrogen atom. [of silica gel] By the radical shown by the above-mentioned general formula [1] concerning this invention, make it replace or a silicon atom is minded for a part of hydrogen atom of the silanol group of the surface (an internal surface and outside surface) of silica gel. By making it replace by the radical shown by the above-mentioned general formula [1] concerning this invention, and making a remaining part or remaining all replace by the alkyl group which may have the substituent It came to complete a header and this invention for denaturation silica gel with the separability of the protein in biological materials (a blood serum, urine, etc.) and a drug useful as a high bulking agent for chromatographies also with the high separability between drugs being obtained.
[0030] Although the silica gel used as a raw material of the denaturation silica gel of this invention will not be limited especially if the function as a bulking agent for chromatographies of the denaturation silica gel of this invention finally obtained is not affected, the silica gel of particle diameter [of 2-50 micrometers] and specific-surface-area 300-800m2/g, the globular form of 50-100A of pore size, or a crushing form is mentioned preferably, for example.
[0031] The general formula introduced through a silicon atom in the denaturation silica gel of this invention [1]
[0032]
[Formula 5]



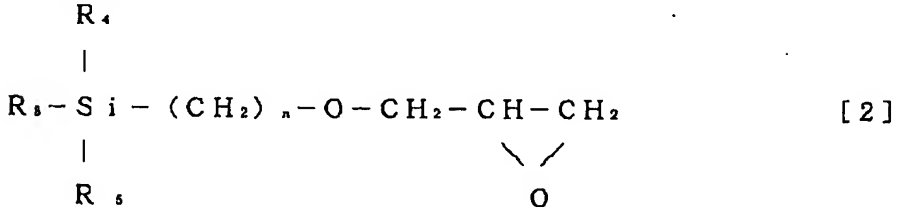
$$-(CH_2)_n-O-CH_2CHCH_2OR_2 \qquad [1]$$
$$\qquad\qquad\quad |$$
$$\qquad\qquad OR_1$$

[0033] Either is a hydrogen atom and R1 and R2 of the radical come out of and shown are another side. - (CH2) mOH is expressed. Here, m expresses two or more integers and expresses the integer of 2-15 preferably. Moreover, in the above-mentioned general formula [1], n expresses three or more integers and expresses the integer of 3-5 preferably.
[0034] as an alkyl group of the alkyl group which may have the substituent introduced through a silicon atom, alkyl groups, such as a methyl group, an ethyl group, a propyl group, butyl, a pentyl radical, a hexyl group, a heptyl radical, an octyl radical, a nonyl radical, and a decyl group, mention in the denaturation silica gel of this invention, for example -- having -- (-- either of the shape of the shape of a straight chain and branching is good.) -- low-grade alkyl groups, such as a methyl group, an ethyl group, a propyl group, butyl, As a substituent, the radical of aromatic series, such as a phenyl group, a tolyl group, and a xylyl group, is mentioned preferably. Moreover, two or more sorts of radicals may be introduced, without limiting the alkyl group which may have the substituent introduced to one kind.
[0035] Although especially the process of the denaturation silica gel of this invention is not limited, the following processes are mentioned, for example.
[0036] - a process 1 -- the **** silica gel first mentioned above and a general formula [2]
[0037]
[Formula 6]



[2]

[0038] R3, R4, and R5 express independently the alkyl group of carbon numbers 1-6, or the alkoxy group of carbon numbers 1-6 among [type, respectively, and at least one of R3, R4, and the R5 is an alkoxy group. Moreover, n expresses three or more integers.] It comes out and the silane coupling agent shown is made to react under acidity or basic catalyst existence.
[0039] Subsequently, if the obtained denaturation silica gel is made to react with a with a carbon numbers of two or more diol compound under acidity or basic catalyst existence, the denaturation silica gel of this invention with which a part or all of a silanol group of a hydrogen atom was replaced by the radical shown by the general formula [1] through a silicon atom will be obtained. [of the internal surface of silica gel and an outside surface]
[0040] This is made to react further with the silane coupling agent which has an alkyl group, and if the alkyl silyl radical combined with the hydroxyl group of the radical shown by the general formula [1] the appropriate back is made to hydrolyze from an acid, the denaturation silica gel of this invention with which it was replaced by the radical a part of hydrogen atom of the silanol group of the internal surface of silica gel and an outside surface is indicated to be by the general formula [1] which starts this invention through a silicon atom, and a remaining part or remaining all was replaced by the
[0041] The denaturation silica gel with which a part of hydrogen atom of a silanol group was replaced through the silicon atom by the radical shown by the general formula [1], and a remaining part or remaining all was replaced by the alkyl group can be manufactured also by the following method again.
[0042] - Make a process 2, i.e., the **** silica gel first mentioned above, and the silane coupling agent which has an alkyl group react.
[0043] Subsequently, they are the bottom of acidity or basic catalyst existence, and a general formula [2] to the obtained denaturation silica gel.
[0044]
[Formula 7]



[2]

[0045] [-- R3, R4, R5, and n are the same as the above among a formula.] It comes out and the silane coupling agent shown is made to react.
[0046] If the obtained denaturation silica gel is made to react to the bottom of acidity or basic catalyst existence with a with a carbon numbers of two or more diol compound, the target denaturation silica gel can be obtained.
[0047] It is as follows when the reagents used in these manufacture method are further explained to details.
[0048] what is not marketed as an example of a silane coupling agent shown by the general formula [2] used by the above-mentioned process 1 and the process 2 although commercial items, such as glycidoxy propyltrimethoxysilane, diethoxy glycidoxypropylmethylsilane, and dimethyl glycidoxy propyl ETOKISHISHIRAN, are mentioned, for example -- for example, Y.Sudo, M.Akiba, T.Sakaki and Y.Takahata, and J.Liq.Chromatogr. -- it can obtain easily by the method of a publication to 17, 1743 (1994), etc.
[0049] Namely, a general formula [3]

[0050]
[Formula 8]

$$CH_2=CH-(CH_2)_{n-2}-OH \qquad [3]$$

[0051] [-- n shows three or more integers among a formula.] Come out, the alcohol and epichlorohydrin which are shown are made to condense under acid catalyst existence, such as concentrated sulfuric acid and boron-trifluoride etherate, and it is a general formula [4].
[0052]
[Formula 9]

$$CH_2=CH-(CH_2)_{n-2}-O-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-CH_2Cl \qquad [4]$$

[0053] [-- n is the same as the above among a formula.] It comes out and considers as the compound shown.
[0054] Subsequently, the compound shown by the general formula [4] is processed by the sodium hydroxide, and it is a general formula [5].
[0055]
[Formula 10]

$$CH_2=CH-(CH_2)_{n-2}-O-CH_2-\underset{\displaystyle O}{\underset{\diagdown\ \diagup}{CH}}-CH_2 \qquad [5]$$

[0056] [-- n is the same as the above among a formula.] It comes out and considers as the epoxy body shown. This epoxy object and a general formula [6]
[0057]
[Formula 11]

$$R_4-\underset{\displaystyle R_5}{\underset{|}{SiH}} \qquad [6]$$

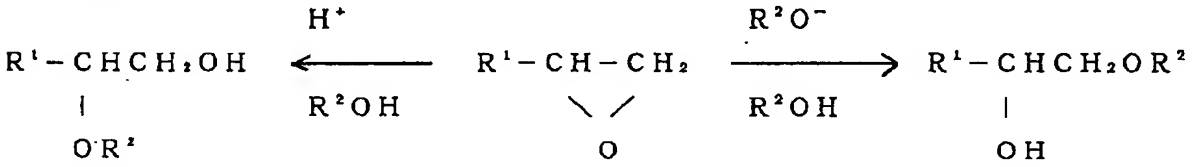
[0058] [-- R3, R4, and R5 are the same as the above among a formula.] The silane coupling agent which comes out and is easily shown by the general formula [2] by making chloroplatinic acid etc. condense as a catalyst in the silane compound shown can be obtained.
[0059] Generally the reaction with the silane coupling agent shown by silica gel and the general formula [2] is performed in an anhydrous solvent like volume on Japan Society for Analytical Chemistry Kanto branch, "high speed liquid chromatography handbook" Maruzen, and Tokyo (1985) p.195 publication. The amount of installation of a silane coupling agent can be changed [the class of silane coupling agent to be used, and] into arbitration from a reaction condition etc.
[0060] In this case, as a basic catalyst used, a pyridine, triethylamine, etc. are mentioned and p-toluenesulfonic acid, 3 fluoride boron etherate, etc. are mentioned as an acid catalyst.
[0061] As a reaction solvent, benzene, toluene, a xylene, etc. are mentioned as a desirable thing. Reaction temperature is a range where 200 degrees C is usually desirable from a room temperature.
[0062] moreover, the process 1 -- reaching -- as a with a carbon numbers of two or more diol compound used in a process 2, ethylene glycol, a propanediol, butanediol, pentanediol, hexandiol, heptane diol, octanediol, nonane diol, the Deccan diol, undecane diol, dodecane diol, tridecane diol, tetradecane diol, pentadecane diol, etc. are specifically mentioned.
[0063] Moreover, as an acid catalyst used in case this diol is made to react, Lewis acid, such as mineral acids, such as a hydrochloric acid, a sulfuric acid, and a phosphoric acid, and 3 fluoride

boron etherate, is mentioned, for example, and a sodium hydroxide, a potassium hydroxide, sodium methylate, sodium ethylate, potassium carbonate, etc. are mentioned as a basic catalyst, for example.

[0064] In addition, you may set making an oxirane (epoxy) compound and alcohol react under acidity or existence of a basic catalyst as indicated in the volume Toshikazu Murahashi and for "new experimental science lecture 14 (I)" Chemical Society of Japan, Maruzen, and Tokyo (1977) p.582, and manufacturing the beta-hydroxy ether to this technical field, and it is known. Moreover, by using an acid catalyst, since the directions of ring breakage of a ring differ on acidity and basic conditions in an unsymmetrical oxirane, by using a basic catalyst, a substituent can be introduced into R1 of the radical shown at the general formula [1] concerning this invention, a hydrogen atom can be introduced into R2, on the other hand, a hydrogen atom can be introduced into R1 of the general formula [1] concerning this invention, and a substituent can be introduced into R2 (following formula 1 reference.).

[0065]

[Formula 1]



[0066] Although a non-solvent may perform the reaction of denaturation silica gel and diol, you may carry out using a suitable solvent. In this case, as a suitable solvent used, dichloromethane, chloroform, 1,2-dichloroethane, 1,1,1-trichloroethane, N.N-dimethylformamide, dimethyl sulfoxide, etc. are mentioned, for example. Moreover, the reaction temperature in this case is usually suitably chosen from the range of 200 degrees C from a room temperature. Thus, a part or all of a silanol group of a hydrogen atom can manufacture easily the denaturation silica gel of this invention replaced by the radical shown by the general formula [1] through a silicon atom. [of the internal surface of silica gel and an outside surface]

[0067] Moreover, as a silane coupling agent which has the alkyl group used by the process 1 and the process 2, they are hexamethyldisilazane, a hexa methyl SHIKUROTORI silazane, an octamethyl cyclo tetra-silazane, and a general formula [7].

[0068]

[Formula 12]

[7]

[0069] R6, R7, and R8 express independently the alkyl group of carbon numbers 1-6, the alkoxy group of carbon numbers 1-6, or a halogen atom among [type, respectively. However, at least one of R6, R7, and the R8 is an alkoxy group or a halogen atom. R9 could express the alkyl group of carbon numbers 1-6, and the aromatic series radical may replace it as a substituent.] It comes out and the silane coupling agent shown is mentioned.

[0070] in addition, as an example of a silane coupling agent shown by the general formula [7] Trimethylchlorosilane, a trimethyl methoxy silane, a trimethyl ethoxy silane, Trimethyl n-propoxysilane, dimethyldichlorosilane, dimethyl dimethoxysilane, Dimethyl diethoxysilane, methyltrichlorosilane, methyl trimetoxysilane, Methyl triethoxysilane, methyl tree n-propoxysilane, triethyl chlorosilicane, Diethyl dichlorosilane, diethyl diethoxysilane, ethyl dimethyl chlorosilicane, Ethyl methyl dichlorosilane, ethyl trichlorosilane, ethyl trimethoxysilane, Ethyltriethoxysilane, tree n-propyl chlorosilicane, G n-propyl dichlorosilane, n-propyl dimethyl chlorosilicane, MECHIRU n-propyl dichlorosilane, n-propyl trichlorosilane, n-

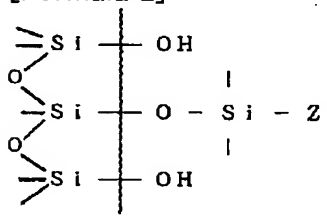
propyltrimethoxysilane, Triisopropyl chlorosilicane, diisopropyl dichlorosilane, isopropyl dimethyl chlorosilicane, Methyl isopropyl dichlorosilane, tree n-butylchlorosilane, G n-butyl methyl chlorosilicane, G n-butyl dichlorosilane, n-buthyldimethyl chlorosilicane, n-butyl methyl dichlorosilane, n-butyl trichlorosilane, i-butyl trichlorosilane, i-butyltrimethoxysilane, G t-butyl methyl chlorosilicane, G t-butyl dichlorosilane, t-buthyldimethyl chlorosilicane, t-butyl trichlorosilane, pentyl trichlorosilane, Pentyl trimethoxysilane, pentyl triethoxysilane, hexyl trichlorosilane, Hexyl methyl dichlorosilane, hexyl trimethoxysilane, hexyl triethoxysilane, benzyl trichlorosilane, a benzyl dimethylethoxy silane, beta-phenethyl trichlorosilane, MECHIRU beta-phenethyl dichlorosilane, etc. are mentioned.

[0071] Although the reaction with the silane coupling agent which has an alkyl group is generally carried out under anhydrous conditions, it is arbitrary. [of adding little water] Moreover, benzene, toluene, a xylene, etc. are mentioned as an example of the suitable reaction solvent used for this reaction. Moreover, as reaction temperature, it is usually the range of 200 degrees C from a room temperature. In addition, if the silane coupling agent which has an alkyl group is made to react after introducing the radical shown in silica gel by the general formula [1], since alkyl silanizing also of the hydroxyl group of the radical shown by the general formula [1] will be carried out, it is necessary to remove by processing the alkyl silyl radical combined with this hydroxyl group in the aqueous solution of the acid containing an acetonitrile, a methanol, ethanol, etc. As an acid used for this processing, a hydrochloric acid, a sulfuric acid, perchloric acid, phosphoric acid, p-toluenesulfonic acid, etc. are mentioned, and the range of reaction temperature is usually 200 degrees C from a room temperature, for example. Thus, the denaturation silica gel with which it was replaced by the radical a part of hydrogen atom of the silanol group of the internal surface of silica gel and an outside surface is easily indicated to be by the general formula [1] concerning this invention, and a remaining part or remaining all was replaced by the alkyl group can be manufactured.

[0072] As a mode in which the radical shown by said general formula [1] through a silicon atom or the alkyl group which may be replaced is introduced into the hydrogen atom of a silanol group, the mode shown, for example in the following formula 2 - a formula 5 is mentioned in the denaturation silica gel of this invention. These are influenced by a class, a reaction condition, etc. of a silane coupling agent to be used.

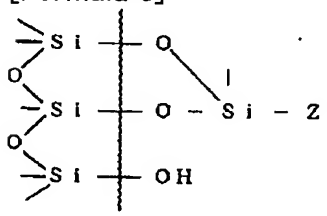
[0073]

[Formula 2]



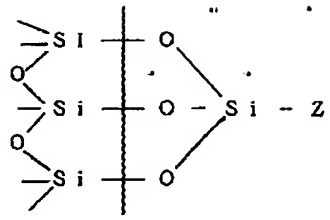
[0074]

[Formula 3]

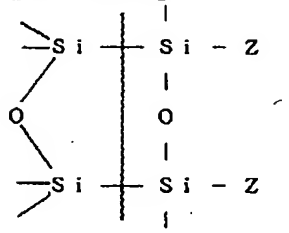


[0075]

[Formula 4]



[0076]
[Formula 5]



[0077] In addition, Z shows said general formula [1] or alkyl group which may be replaced. It is characterized by the bulking agent for chromatographies of this invention coming to contain the denaturation silica gel of **** this invention mentioned above.
[0078] Although the bulking agent which comes to contain the denaturation silica gel of this invention can be used like general opposition column packing material, it is effective in analysis and concentration of the component in the biological material which contains especially a protein component so much.
[0079] It is characterized by the column for chromatographies of this invention coming to fill up the bulking agent for chromatographies of above-mentioned this invention.
[0080] The column for chromatographies of this invention is obtained the bore (phi) of 1-10mm by filling up 2-6mm, a length of 10-500mm, and the 10-300mm desirable column made from stainless steel with the denaturation silica gel of this invention according to conventional methods, such as slurry method, preferably.
[0081] the solvent with which protein does not denaturalize as a mobile phase at the time of analyzing the component in a biological material using the bulking agent for chromatographies of this invention -- an organic solvent content is preferably mentioned for 30% or less of acetonitrile, isopropyl alcohol, a tetrahydrofuran, ethanol, etc. aqueous intermediation, etc., and the mixed solvent or water of an acetonitrile and water is especially mentioned as a desirable thing. Moreover, close buffers usually used in this field, such as phosphate, acetate, formate, a tartrate, and citrate, may be in a mobile phase if needed in addition to an organic solvent or water. Moreover, it is also arbitrary by performing gradient actuation to change the concentration of an organic solvent one by one. Moreover, especially if pH of a mobile phase is the range which can analyze the quality of the specified substance, it will not be limited, but if a column life is taken into consideration, it will be mentioned as a range where pH 2-7.5 is desirable.
[0082] The rate of flow at the time of elution is usually preferably chosen from the range of 0.5 - 2 ml/min suitably 0.1 to 5 ml/min in the analytical method using the bulking agent of this invention. 0-60 degrees C of column temperature are usually preferably chosen from the range of 20-50 degrees C suitably.
[0083] By analyzing under the above-mentioned **** conditions, it becomes possible to analyze easily and simple, without carrying out deproteination processing of the components (for example, phenobarbital, carbamazepine, phenytoin, lidocaine, primidone, imipramine, indomethacin, a chloramphenicol, trimethoprim, nitrazepam, furosemide, ethenzamide, salicylamide, ibuprofen, tolbutamide, acetaminophen, etc.), such as a drug in the biological material which contains a protein component in large quantities. That is, by introducing the substituent shown in silica gel by the general formula [1] concerning this invention, macromolecules, such as protein, are bypassed without denaturalizing and are discharged as they are out of a column by the end hydroxyl group and ether substructure in the radical shown by the general formula [1] concerning

this invention. According to a hydrophobic difference, separation elution of the compounds with a molecule small on the other hand comparatively (drug etc.) is carried out by the radical shown by the general formula [1] concerning this invention, or the radical and alkyl group which are shown by the general formula [1] concerning this invention. In addition, separability, such as a drug, has a high direction at the time of using the bulking agent which comes to contain the denaturation silica gel of this invention with which both the radical shown by the general formula [1] concerning this invention and the alkyl group were introduced in this case.

[0084] The bulking agent which comes to contain the denaturation silica gel of this invention can be bypassed again, without protein denaturalizing, and low molecular weight compounds, such as a drug, can be used also as pretreatment column packing material for deproteination [being held].

[0085] That is, while condensing minor constituents, such as a drug in a biological material, by letting a biological material pass in the column filled up with the denaturation silica gel of this invention as a bulking agent, a protein component is eliminated out of a column. Next, it analyzes by leading minor constituents, such as a drug condensed within the column of this invention, to a common opposition column (column for analysis). It is also arbitrary to use the technique of switching automatically the passage of the mobile phase known as a column switching method at this time using a high-pressure roppo bulb etc.

[0086] A drug with the low concentration of the biological material middle class etc. can be analyzed to high sensitivity by using as the pretreatment column for deproteination the column filled up with the denaturation silica gel of this invention as a bulking agent as mentioned above, and using it combining these, using a common opposition system column as the column for analysis. Of course, it cannot be overemphasized that the column filled up with the denaturation silica gel of this invention as a bulking agent may be used as a column for analysis as it is.

[0087] Next, although an example and the example of reference explain this invention concretely, this invention is not limited at all by these.

[0088]

[Example]

Example 1 of reference Composition of the silica gel which has 3-glycidoxy propyl silyl radical (when silica gel with about 80A [of pore size] and a mean particle diameter of 5 micrometers is used)

About 80A of pore size, mean particle diameter 5-micrometer globular form silica gel It is toluene to 20g. Water after adding 400ml and removing water with azeotropy 0.82ml, 3-glycidoxy propyltrimethoxysilane 19.67g and triethylamine 6ml was added and it was made to flow back for 8 hours. The obtained silica gel is separated after cooling and it is toluene. 400ml, methanol 400ml, water 1000ml, acetone 400ml and methanol Silica gel which dries at 85 degrees C with a vacuum dryer, and has 3-glycidoxy propyl silyl radical after washing in 400ml order 25.8g was obtained.

[0089] Example 2 of reference Composition of the silica gel which has 2 and 3-dihydroxy propoxy propyl silyl radical (when silica gel with about 80A [of pore size] and a mean particle diameter of 5 micrometers is used)

Denaturation silica gel obtained in the example 1 of reference It is 0.7% perchloric acid aqueous solution to 4g. 180ml and acetonitrile 20ml was added and it was made to flow back for 6 hours. The obtained silica gel is separated after cooling and it is a methanol. 200ml, water 500ml, acetone 200ml and methanol Silica gel which dries at 85 degrees C with a vacuum dryer, and has 2 and 3-dihydroxy propoxy propyl silyl radical after washing in 200ml order 2.7g was obtained.

[0090] Example 3 of reference Composition of the silica gel which has 5 and 6-dihydroxy hexyl silyl radical (when silica gel with about 80A [of pore size] and a mean particle diameter of 5 micrometers is used)

About 80A of pore size, mean particle diameter 5-micrometer globular form silica gel 7.5g, toluene 150ml, water 0.46ml, 5, 6-epoxy hexyl trimethoxysilane 6.9g, triethylamine Silica gel which processes like the example 1 of reference using 2.25ml, and has 5 and 6-epoxy hexyl silyl radical 9.6g was obtained. This silica gel 8.0g, 0.7% perchloric acid aqueous solution 360.45ml and acetonitrile Silica gel which processes like the example 2 of reference using 40.05ml, and has 5

and 6-dihydroxy hexyl silyl radical 7.9g was obtained.
[0091] Example 1 Composition of the silica gel which has a 2-(2-hydroxy ethoxy)-3-hydroxy propoxy propyl silyl radical (when silica gel with about 80A [of pore size] and a mean particle diameter of 5 micrometers is used)
Denaturation silica gel obtained in the example 1 of reference It is chloroform to 3.5g. 5ml, ethylene glycol 4.34g and BF3, and 200.2 g Et were added, and it was made to flow back for 4 hours. The obtained silica gel is separated after cooling and it is a methanol. 200ml, water 500ml, acetone Silica gel which dries at 85 degrees C with a vacuum dryer, and has a 2-(2-hydroxy ethoxy)-3-hydroxy propoxy propyl silyl radical after washing in order (200ml and methanol 200ml) 3.3g was obtained.
[0092] Example 2 Composition of the silica gel which has a 2-(2-hydroxy ethoxy)-3-hydroxy propoxy propyl silyl radical and a trimethylsilyl radical (when silica gel with about 80A [of pore size] and a mean particle diameter of 5 micrometers is used)
Denaturation silica gel obtained in the example 1 It is toluene to 2.3g. 11.5ml and 1, 1, 1, 3 and 3, 3-hexamethyldisilazane 4.6ml was added and it was made to flow back for 8 hours. The obtained silica gel is separated after cooling and it is toluene. 200ml, methanol 200ml, water 500ml, acetone 200ml and methanol After washing in 200ml order, it dried at 85 degrees C with the vacuum dryer. It is 0.7% perchloric acid aqueous solution to this silica gel. 93.15ml and acetonitrile 10.35ml was added and it was made to flow back for 2 hours. The obtained silica gel is separated after cooling and it is a methanol. 200ml, water 500ml, acetone 200ml and methanol After washing in 200ml order, it dried at 85 degrees C with the vacuum dryer, and silica gel 1.7g which has a 2-(2-hydroxy ethoxy)-3-hydroxy propoxy propyl silyl radical and a trimethylsilyl radical was obtained.
[0093] Example 3 Composition of the silica gel which has a 2-(4-hydroxy butoxy)-3-hydroxy propoxy propyl silyl radical (when silica gel with about 80A [of pore size] and a mean particle diameter of 5 micrometers is used)
Denaturation silica gel obtained in the example 1 of reference 3.5g, chloroform 5ml, butanediol 6.31g and silica gel which processes like an example 1 using BF3 and 200.2 g Et, and has a 2-(4-hydroxy butoxy)-3-hydroxy propoxy propyl silyl radical 3.3g was obtained.
[0094] Example 4 Composition of the silica gel which has a 2-(4-hydroxy butoxy)-3-hydroxy propoxy propyl silyl radical and a trimethylsilyl radical (when silica gel with about 80A [of pore size] and a mean particle diameter of 5 micrometers is used)
Denaturation silica gel obtained in the example 3 It is toluene to 2.2g. 11ml and 1, 1, 1, 3 and 3, 3-hexamethyldisilazane Silica gel which performs the same processing as an example 2 using 4.4ml, and has a 2-(4-hydroxy butoxy)-3-hydroxy propoxy propyl silyl radical and a trimethylsilyl radical 1.8g was obtained.
[0095] Example 5 Composition of the silica gel which has a 2-(6-hydroxy hexyloxy)-3-hydroxy propoxy propyl silyl radical (when silica gel with about 80A [of pore size] and a mean particle diameter of 5 micrometers is used)
Denaturation silica gel obtained in the example 1 of reference 3.5g, chloroform 5ml, hexandiol 8.27g, and silica gel that performs the same processing as an example 1 using BF3 and 200.2 g Et, and has a 2-(6-hydroxy hexyloxy)-3-hydroxy propoxy propyl silyl radical 3.3g was obtained.
[0096] Example 6 Composition of the silica gel which has a 2-(6-hydroxy hexyloxy)-3-hydroxy propoxy propyl silyl radical and a trimethylsilyl radical (when pore size about 80A and silica gel with a mean particle diameter of 5 micrometers are used)
Denaturation silica gel obtained in the example 5 It is toluene to 2.3g. 11.5ml and 1, 1, 1, 3 and 3, 3-hexamethyldisilazane Silica gel which performs the same processing as an example 2 using 4.6ml, and has a 2-(6-hydroxy hexyloxy)-3-hydroxy propoxy propyl silyl radical and a trimethylsilyl radical 1.9g was obtained.
[0097] Example 4 of reference Composition of the silica gel which has a 2-butoxy 3-hydroxy propoxy propyl silyl radical (when silica gel with about 80A [of pore size] and a mean particle diameter of 5 micrometers is used)
Denaturation silica gel obtained in the example 1 of reference 3.5g, chloroform Silica gel which performs the same processing as an example 1 using 5ml, butanol 5.19g, and 200.2 g BF3 and

</FONT>Et, and has a 2-butoxy 3-hydroxy propoxy propyl silyl radical 3.4g was obtained.
[0098] Example 5 of reference Composition of the silica gel which has 3-glycidoxy propyl silyl radical (when silica gel with about 60A [of pore size] and a mean particle diameter of 5 micrometers is used)
Globular form silica gel with about 60A [of pore size], and a mean particle diameter of 5 micrometers 20g, toluene 400ml, water 1.03ml, glycidoxy propyltrimethoxysilane 26.14g, triethylamine Silica gel which performs the same processing as the example 1 of reference using 6ml, and has 3-glycidoxy propyl silyl radical 27.7g was obtained.
[0099] Example 6 of reference Composition of the silica gel which has 2 and 3-dihydroxy propoxy propyl silyl radical (when silica gel with about 60A [of pore size] and a mean particle diameter of 5 micrometers is used)
Denaturation silica gel obtained in the example 5 of reference 3.5g, 0.7% perchloric acid aqueous solution 157.5ml and acetonitrile Silica gel which performs the same processing as the example 2 of reference using 17.5ml, and has 2 and 3-dihydroxy propoxy propyl silyl radical 3.4g was obtained.
[0100] Example 7 of reference Composition of the silica gel which has 5 and 6-dihydroxy hexyl silyl radical (when silica gel with about 60A [of pore size] and a mean particle diameter of 5 micrometers is used)
Globular form silica gel with about 60A [of pore size], and a mean particle diameter of 5 micrometers 7.5g, toluene 150ml, water 0.59ml, 5, 6-epoxy hexyl trimethoxysilane 13.0g, triethylamine Silica gel which performs the same processing as the example 1 of reference using 2.25ml, and has 5 and 6-epoxy hexyl silyl radical 10.6g was obtained. This silica gel 8.7g, 0.7% perchloric acid aqueous solution 390.6ml and acetonitrile Silica gel which performs the same processing as the example 2 of reference using 43.4ml, and has 5 and 6-dihydroxy hexyl silyl radical 8.5g was obtained.
[0101] Example 8 of reference Composition of the silica gel which has 2-(3, 4-dihydroxy cyclohexyl) ethyl silyl radical (when silica gel with about 60A [of pore size] and a mean particle diameter of 5 micrometers is used)
Globular form silica gel with about 60A [of pore size], and a mean particle diameter of 5 micrometers 5.0g, toluene 100ml, 2.0ml of water, 2-(3, 4-epoxycyclohexyl) ethyl trimethoxysilane 5.0g, triethylamine Silica gel which performs the same processing as the example 1 of reference using 1.50ml, and has 2-(3, 4-epoxycyclohexyl) ethyl silyl radical 6.2g was obtained. This silica gel 2.0g, 0.7% perchloric acid aqueous solution Silica gel which performs the same processing as the example 2 of reference using 80.0ml and acetonitrile 20.0ml, and has 2-(3, 4-dihydroxy cyclohexyl) ethyl silyl radical 1.9g was obtained.
[0102] Example 7 Composition of the silica gel which has a 2-(2-hydroxy ethoxy)-3-hydroxy propoxy propyl silyl radical (when silica gel with about 60A [of pore size] and a mean particle diameter of 5 micrometers is used)
Denaturation silica gel obtained in the example 5 of reference 3.5g, chloroform 5.0ml, ethylene glycol 5.65g and silica gel which performs the same processing as an example 1 using BF3 and 200.26 g Et, and has a 2-(2-hydroxy ethoxy)-3-hydroxy propoxy propyl silyl radical 3.4g was obtained.
[0103] Example 8 Composition of the silica gel which has a 2-(2-hydroxy ethoxy)-3-hydroxy propoxy propyl silyl radical and a trimethylsilyl radical (when silica gel with about 60A [of pore size] and a mean particle diameter of 5 micrometers is used)
Denaturation silica gel obtained in the example 7 It is toluene to 2.3g. 11.5ml and 1, 1, 1, 3 and 3, 3-hexamethyldisilazane Silica gel which performs the same processing as an example 2 using 4.6ml, and has a 2-(2-hydroxy ethoxy)-3-hydroxy propoxy propyl silyl radical and a trimethylsilyl radical 1.9g was obtained.
[0104] Example 9 Composition of the silica gel which has a 2-(4-hydroxy butoxy)-3-hydroxy propoxy propyl silyl radical (when silica gel with about 60A [of pore size] and a mean particle diameter of 5 micrometers is used)
Denaturation silica gel obtained in the example 5 of reference 3.5g, chloroform 5ml, butanediol 8.20g and silica gel which performs the same processing as an example 1 using BF3 and 200.26 g

Et, and has a 2-(4-hydroxy butoxy)-3-hydroxy propoxy propyl silyl radical 3.3g was obtained.
[0105] Example 10 Composition of the silica gel which has a 2-(4-hydroxy butoxy)-3-hydroxy propoxy propyl silyl radical and a trimethylsilyl radical (when silica gel with about 60A [of pore size] and a mean particle diameter of 5 micrometers is used)
Denaturation silica gel obtained in the example 9 It is toluene to 2.3g. 11.5ml and 1, 1, 1, 3 and 3, 3-hexamethyldisilazane Silica gel which performs the same processing as an example 2 using 2.3ml, and has a 2-(4-hydroxy butoxy)-3-hydroxy propoxy propyl silyl radical and a trimethylsilyl radical 1.9g was obtained.
[0106] Example 11 Composition of the silica gel which has a 2-(6-hydroxy hexyloxy)-3-hydroxy propoxy propyl silyl radical (when silica gel with about 60A [of pore size] and a mean particle diameter of 5 micrometers is used)
Denaturation silica gel obtained in the example 5 of reference 3.5g, chloroform 5ml, hexandiol 10.75g, and silica gel that performs the same processing as an example 1 using BF3 and 200.26 g Et, and has a 2-(6-hydroxy hexyloxy)-3-hydroxy propoxy propyl silyl radical 3.3g was obtained.

[0107] Example 12 Composition of the silica gel which has a 2-(6-hydroxy hexyloxy)-3-hydroxy propoxy propyl silyl radical and a trimethylsilyl radical (when silica gel with about 60A [of pore size] and a mean particle diameter of 5 micrometers is used)
Denaturation silica gel obtained in the example 11 It is toluene to 2.4g. 12ml and 1, 1, 1, 3 and 3, 3-hexamethyldisilazane Silica gel which performs the same processing as an example 2 using 4.8ml, and has a 2-(6-hydroxy hexyloxy)-3-hydroxy propoxy propyl silyl radical and a trimethylsilyl radical 2.0g was obtained.
[0108] Example 13 Composition of the silica gel which has a 2-(6-hydroxy hexyloxy)-3-hydroxy propoxy propyl silyl radical and a dimethylsilyl radical (when silica gel with about 60A [of pore size] and a mean particle diameter of 5 micrometers is used)
Denaturation silica gel obtained in the example 11 It is toluene to 4.0g. 20ml and 1, 1, 3, 3 and 5, and 5-hexa methyl SHIKUROTORI silazane Silica gel which performs the same processing as an example 2 using 8.4g, and has a 2-(6-hydroxy hexyloxy)-3-hydroxy propoxy propyl silyl radical and a dimethylsilyl radical 3.7g was obtained.
[0109] Example 14 The stainless steel column for HPLC with a bore [of 4.6mm] and a length of 50mm was filled up with the denaturation silica gel manufactured in the recovery test examples 1, 3, and 5 and the examples 2, 3, and 4 of reference of cow serum albumin with slurry method. Cow serum albumin (it is hereafter written as BSA.) was processed on the analysis conditions shown below using each column, and the recovery was searched for. The result is shown in a table 1.
[0110] [Analysis conditions]
mobile phase: -- 100mM phosphate buffer solution (pH=6.9) / acetonitrile =90/10 (v/v) rate-of-flow: -- 0.5 ml/min measurement temperature: -- 30-degree-C detection wavelength: -- 254nm sample: -- liquid injection rate: which dissolved BSA 15mg/ml in the above-mentioned mobile phase -- 20microl [0111]
表 1

充填剤の種類	BSA回収率%
参考例２のシリカゲル	97.4
参考例３のシリカゲル	97.2
実施例１のシリカゲル	95.8
実施例３のシリカゲル	95.8
実施例５のシリカゲル	96.4
参考例４のシリカゲル	49.1

[0112] From the result of a table 1, the BSA recovery in the column filled up with the

denaturation silica gel of this invention of examples 1, 3, and 5 as a bulking agent showed good BSA recovery like the column filled up with the bulking agent with which the portion of the hydrophilic radical obtained, the conventional bulking agents 2 and 3, i.e., examples of reference, has the conventional diol form. In addition, the peak configuration of BSA of the chromatogram obtained in the column filled up with the denaturation silica gel of examples 1, 3, and 5 as a bulking agent was a configuration with it. [there is little tailing and better than it which is obtained in the column filled up with the bulking agent of the conventional examples 2 and 3 of reference] Moreover, when the BSA recovery at the time of using the denaturation silica gel of an example 3 as a bulking agent is compared with BSA recovery when this and a carbon number use the silica gel (however, the hydroxyl group is introduced into the end only for one.) of the example 4 of reference with which the qualification silyl radical of the same number was introduced as a bulking agent, it turns out that the direction at the time of using denaturation silica gel of an example 3 as a bulking agent shows very high recovery. In order to acquire good protein recovery from this, it turns out that it is required to introduce the hydroxyl group into the end of R1 of the radical shown in the bulking agent concerning this invention by the general formula [1] like.

[0113] Example 15 Analysis of a uracil, benzene, and naphthalene was performed on condition that the following using each column prepared in a uracil, benzene, and the assay example 14 of naphthalene, and each holding time was found. The result is shown in a table 2.

[Analysis conditions]

mobile phase: -- CH3 CN/H2O=30 / 70 (v/v) rate-of-flow: -- 0.5 ml/min measurement temperature: -- 30-degree-C detection wavelength: -- 254nm sample: -- uracil 0.77mg and benzene 145microl and naphthalene 20mg -- 60%CH3CN aqueous solution liquid injection rate: dissolved in 100ml -- 2microl [0114]

表 2

充填剤の種類	保持時間(分)		
	ウラシル	ベンゼン	ナフタレン
参考例2のシリカゲル	1.37	1.83	2.63
参考例3のシリカゲル	1.36	2.28	4.01
実施例1のシリカゲル	1.36	1.88	2.84
実施例3のシリカゲル	1.37	2.04	3.35
実施例5のシリカゲル	1.39	2.28	4.16
参考例4のシリカゲル	1.37	2.57	4.85

[0115] The result of a table 2 shows that a uracil, benzene, and naphthalene may be separated good like the column filled up with the bulking agent of the examples 2, 3, and 4 of reference by using the column filled up with the denaturation silica gel of this invention of examples 1, 3, and 5 as a bulking agent. Moreover, in the case of the bulking agent concerning this invention, as for by changing the carbon number of the methylene chain of R1 of the radical shown by the general formula [1] concerning this invention, the holding time of benzene and naphthalene also shows that it can adjust easily (example 1: a carbon number 2, the example 3:carbon number 4, example 5:carbon number 6).

[0116] Example 16 Analysis of phenobarbital, carbamazepine, and phenytoin was performed on condition that the following using each column prepared in phenobarbital, carbamazepine, and the assay example 14 of phenytoin, and each holding time was found. The result is shown in a table 3.

[Analysis conditions]

mobile phase: -- 100mM phosphate buffer solution (pH=6.9) / acetonitrile =90/10 (v/v) rate-of-

flow: -- 0.5 ml/min measurement temperature: -- 30-degree-C detection wavelength: -- 254nm sample: -- phenobarbital 2mg and carbamazepine 0.5mg and phenytoin liquid injection rate: which dissolved 4mg in the 100ml of the above-mentioned mobile phases -- 20microl [0117]

表 3

充填剤の種類	保持時間(分)		
	ﾌｪﾉﾊﾞﾙﾋﾞﾀｰﾙ	ｶﾙﾊﾞﾏｾﾞﾋﾟﾝ	ﾌｪﾆﾄｲﾝ
参考例2のシリカゲル	2.87	5.14	5.86
参考例3のシリカゲル	4.02	9.13	10.65
実施例1のシリカゲル	3.13	6.08	7.05
実施例3のシリカゲル	3.45	6.81	8.66
実施例5のシリカゲル	3.87	7.84	10.77
参考例4のシリカゲル	4.12	8.62	12.39

[0118] The result of a table 3 shows that the column filled up with the denaturation silica gel of this invention of examples 1, 3, and 5 as a bulking agent has separated phenobarbital, carbamazepine, and phenytoin good. In the column filled up with the bulking agent (bulking agent with which the portion of the hydrophilic radical introduced into the internal surface and outside surface of silica gel has the conventional diol form) of the examples 2 and 3 of reference which are the conventional bulking agents on the other hand, a part of peak of carbamazepine and phenytoin was not able to lap on the same conditions, and these were not able to be separated good. It is thought that the three above-mentioned sorts of drugs are separable from these things good since the radical shown in the bulking agent filled up with the denaturation silica gel of examples 1, 3, and 5 by the general formula [1] concerning this invention is introduced. Moreover, as for by changing the carbon number of the methylene chain of R1 of the radical shown by the general formula [1] concerning this invention from the result of a table 3, the holding time of these drugs also shows that it can adjust easily (example 1: a carbon number 2, the example 3:carbon number 4, example 5:carbon number 6).

[0119] Example 17 The stainless steel column for HPLC with a bore [of 4.6mm] and a length of 50mm was filled up with the denaturation silica gel manufactured in the recovery test examples 2, 4, and 6 of cow serum albumin with slurry method. The recovery of BSA was searched for on the same conditions as an example 14 using these columns. The result is shown in a table 4.

[0120]

表 4

充填剤の種類	BSA回収率%
実施例2のシリカゲル	94.5
実施例4のシリカゲル	93.0
実施例6のシリカゲル	87.8

[0121] It turns out that the column filled up with the denaturation silica gel of this invention which introduced the radical shown in silica gel by the general formula [1] concerning this invention from the result of a table 4 and the trimethylsilyl radical as a bulking agent shows good BSA recovery.

[0122] Example 18 Analysis of a uracil, benzene, and naphthalene was performed on the same conditions as an example 15 using each column prepared and carried out in a uracil, benzene, and the assay example 17 of naphthalene, and each holding time was found. The result is shown

in a table 5. "
[0123]
表 5

充填剤の種類	保持時間(分)		
	ウラシル	ベンゼン	ナフタレン
実施例2のシリカゲル	1.45	2.13	3.45
実施例4のシリカゲル	1.39	2.35	4.22
実施例6のシリカゲル	1.37	2.59	5.05

[0124] The column filled up with the denaturation silica gel of this invention prepared in the examples 2, 4, and 6 as a bulking agent from the result of a table 5 shows that a uracil, benzene, and naphthalene are separable good. Moreover, when the bulking agent (examples 2, 4, and 6) concerning this invention into which the trimethylsilyl radical was introduced instead of the bulking agent (examples 1, 3, and 5) applied to this invention into which the trimethylsilyl radical is not introduced from the comparison of the data of a table 2 and a table 5 is used, it also turns out that the holding time of each compound becomes long. Moreover, even if it is the case where a trimethylsilyl radical lives together from the result of a table 5, by changing the prime factor of the methylene chain of R1 of the radical shown by the general formula [1] concerning this invention also shows that the holding time of benzene and naphthalene can be adjusted easily (example 2: a carbon number 2, the example 4:carbon number 4, example 6:carbon number 6).
[0125] Example 19 Analysis of phenobarbital, carbamazepine, and phenytoin was performed on the same conditions as an example 16 using each column prepared in phenobarbital, carbamazepine, and the assay example 17 of phenytoin, and each holding time was found. The result is shown in a table 6.
[0126]
表 6

充填剤の種類	保持時間(分)		
	ﾌｪﾉﾊﾞﾙﾋﾞﾀｰﾙ	ｶﾙﾊﾞﾏｾﾞﾋﾟﾝ	ﾌｪﾆﾄｲﾝ
実施例2のシリカゲル	3.84	7.65	9.95
実施例4のシリカゲル	4.54	9.10	13.11
実施例6のシリカゲル	4.51	9.86	14.61

[0127] The result of a table 6 shows that each drug may be separated good by using the column filled up with the denaturation silica gel of this invention prepared in the examples 2, 4, and 6 as a bulking agent. Moreover, the holding time of each drug also understands [the direction at the time of using the bulking agent (examples 2, 4, and 6) concerning this invention into which the trimethylsilyl radical was introduced] becoming long rather than the case where the bulking agent (examples 1, 3, and 5) applied to this invention into which the trimethylsilyl radical is not introduced from the comparison of the data of a table 3 and a table 6 is used. Moreover, from the result of a table 6, even when a trimethylsilyl radical is introduced, by changing the carbon number of the methylene chain of R1 of the radical shown by the general formula [1] concerning this invention also shows that the holding time of each drug can be adjusted easily (example 2: a carbon number 2, the example 4:carbon number 4, example 6:carbon number 6).
[0128] Example 20 The stainless steel column for HPLC with a bore [of 4.6mm] and a length of

50mm was filled up with the silica gel manufactured in the recovery test examples 7, 9, and 11 and the examples 6, 7, and 8 of reference of cow serum albumin with slurry method. The recovery of BSA was searched for on the same conditions as an example 14 using each column. The result is shown in a table 7.

[0129]

表 7

充填剤の種類	BSA回収率%
参考例６のシリカゲル	92.0
参考例７のシリカゲル	91.0
参考例８のシリカゲル	88.6
実施例７のシリカゲル	94.2
実施例９のシリカゲル	93.9
実施例１１のシリカゲル	94.3

[0130] The result of a table 7 shows that the BSA recovery in the column filled up with the denaturation silica gel of this invention of examples 7, 9, and 11 as a bulking agent is good like it in the column filled up with the bulking agent of the conventional diol form, i.e., the bulking agent prepared in the examples 6, 7, and 8 of reference. Moreover, the peak configuration of BSA of the chromatogram obtained in the column filled up with the denaturation silica gel of examples 7, 9, and 11 as a bulking agent was a good configuration with little tailing.

[0131] Example 21 Analysis of a uracil, benzene, and naphthalene was performed on the same conditions as an example 15 using each column prepared in a uracil, benzene, and the assay example 20 of naphthalene, and each holding time was found. The result is shown in a table 8.

[0132]

表 8

充填剤の種類	保持時間(分)		
	ウラシル	ベンゼン	ナフタレン
参考例６のシリカゲル	1.34	1.93	3.00
参考例７のシリカゲル	1.38	2.29	4.31
参考例８のシリカゲル	1.26	1.77	2.50
実施例７のシリカゲル	1.32	2.06	3.50
実施例９のシリカゲル	1.33	2.16	3.85
実施例１１のシリカゲル	1.33	2.52	5.08

[0133] From the result of a table 8, the column filled up with the denaturation silica gel of this invention of examples 7, 9, and 11 as a bulking agent shows that a uracil, benzene, and naphthalene are separable good like the column filled up with the conventional bulking agent (bulking agent with which the portion of the hydrophilic radical introduced into the internal surface and outside surface of a silica has a form of the conventional diol) of the examples 6, 7, and 8 of reference. Moreover, as for by changing the carbon number of the methylene chain of R1 of the radical shown by the general formula [1] which starts this invention in the bulking agent concerning this invention, the holding time also shows that it can adjust easily (example 7: a carbon number 2, the example 9:carbon number 4, example 11:carbon number 6).

[0134] Example 22 Analysis of phenobarbital, carbamazepine, and phenytoin was performed on

the same conditions as an example 16 using each column prepared in phenobarbital, carbamazepine, and the assay example 20 of phenytoin, and each holding time was found. The result is shown in a table 9.

[0135]

表 9

充填剤の種類	保持時間(分)		
	ﾌｪﾉﾊﾞﾙﾋﾞﾀｰﾙ	ｶﾙﾊﾞﾏｾﾞﾋﾟﾝ	ﾌｪﾆﾄｲﾝ
参考例６のシリカゲル	3.29	5.54	7.22
参考例７のシリカゲル	4.51	10.25	13.01
参考例８のシリカゲル	2.27	9.56	8.99
実施例７のシリカゲル	3.81	6.95	9.54
実施例９のシリカゲル	3.97	7.19	10.12
実施例１１のシリカゲル	4.67	8.70	13.56

[0136] The result of a table 9 shows that it may dissociate better than [the case where the column filled up with the bulking agent of the examples 6, 7, and 8 of reference for each drug is used with the column filled up with the denaturation silica gel of this invention of examples 7, 9, and 11 as a bulking agent, and] an EQC. When the column especially filled up with the bulking agent of an example 11 is used, it turns out that carbamazepine and phenytoin may be separated more into fitness from the case where the column filled up with the bulking agent of the examples 6, 7, and 8 of reference is used. Moreover, as for by changing the carbon number of the methylene chain of R1 of the radical shown by the general formula [1] which starts this invention from the result of a table 9, the holding time of each drug also shows that it can adjust easily (example 1: a carbon number 2, the example 3:carbon number 4, example 5:carbon number 6).

[0137] The stainless steel column for HPLC with a bore [of 4.6mm] and a length of 50mm was filled up with the silica gel manufactured in the recovery test examples 8, 10, 12, and 13 of example 23 cow serum albumin with slurry method. The recovery of BSA was searched for on the same conditions as an example 14 using each column. The result is shown in a table 10.

[0138]

表 １０

充填剤の種類	BSA回収率%
実施例８のシリカゲル	94.6
実施例１０のシリカゲル	91.7
実施例１２のシリカゲル	86.9
実施例１３のシリカゲル	88.4

[0139] The result of a table 10 shows that each BSA recovery in the column filled up with the denaturation silica gel of examples 8, 10, 12, and 13 as a bulking agent is good.

[0140] Example 24 Analysis of a uracil, benzene, and naphthalene was performed on the same conditions as an example 15 using each column prepared in a uracil, benzene, and the assay example 23 of naphthalene, and each holding time was found. The result is shown in a table 11.

[0141]

表 11

充填剤の種類	保持時間(分)		
	ウラシル	ベンゼン	ナフタレン
実施例8のシリカゲル	1.32	2.28	4.18
実施例10のシリカゲル	1.34	2.50	4.90
実施例12のシリカゲル	1.34	2.84	6.26
実施例13のシリカゲル	1.29	2.67	5.26

[0142] Even when any of the column filled up with the denaturation silica gel of this invention of examples 8, 10, and 12 as a bulking agent (the trimethylsilyl radical is introduced) and the column filled up with the denaturation silica gel of this invention of an example 13 as a bulking agent (the dimethylsilyl radical is introduced) are used from the result of a table 11, it turns out that a uracil, benzene, and naphthalene may be separated good. Moreover, the direction which used the bulking agent (bulking agent of examples 8, 10, 12, and 13) concerning this invention into which the trimethylsilyl radical etc. was introduced from the comparison of the data of a table 8 and a table 11 as compared with the bulking agent (bulking agent of examples 7, 9, and 11) concerning this invention into which neither the trimethylsilyl radical nor the dimethylsilyl radical is introduced also understands that the holding time of each compound becomes long. Moreover, as for by changing the carbon number of the methylene chain of R1 of the radical shown by the general formula [1] concerning this invention from the result of a table 11, the holding time also shows that it can adjust easily (example 8: a carbon number 2, the example 10:carbon number 4, example 12:carbon number 6).

[0143] Example 25 Analysis of phenobarbital, carbamazepine, and phenytoin was performed on the same conditions as an example 16 using each column prepared in phenobarbital, carbamazepine, and the assay example 23 of phenytoin, and each holding time was found. The result is shown in a table 12.

[0144]

表 12

充填剤の種類	保持時間(分)		
	ﾌｪﾉﾊﾞﾙﾋﾞﾀｰﾙ	ｶﾙﾊﾞﾏｾﾞﾋﾟﾝ	ﾌｪﾆﾄｲﾝ
実施例8のシリカゲル	4.85	8.76	13.37
実施例10のシリカゲル	5.00	9.53	15.18
実施例12のシリカゲル	6.16	11.33	20.55
実施例13のシリカゲル	5.20	9.11	15.81

[0145] Even if it uses any of the column filled up with the denaturation silica gel of this invention of examples 8, 10, and 12 as a bulking agent (the trimethylsilyl radical is introduced), and the column filled up with the denaturation silica gel of this invention of an example 13 as a bulking agent (the dimethylsilyl radical is introduced) from the result of a table 12, it turns out that each drug may be separated good. From the comparison of the data of a table 9 and a table 12 rather than moreover, the case where the bulking agent (bulking agent of examples 7, 9, and 11) concerning this invention into which neither the trimethylsilyl radical nor the dimethylsilyl radical is introduced is used The direction at the time of using the bulking agent (bulking agent of examples 8, 10, 12, and 13) concerning this invention into which the trimethylsilyl radical etc. was

introduced If the column filled up with that the holding time of each drug can be lengthened and the denaturation silica gel of examples 8, 10, 12, and 13 as a bulking agent is used The case where the column filled up with the conventional bulking agent (bulking agent of the examples 6, 7, and 8 of reference) is used shows that carbamazepine and phenytoin are also more separable into fitness. Moreover, the thing (example 8: a carbon number 2, the example 10:carbon number 4, example 12:carbon number 6) for which the holding time can be easily adjusted by changing the carbon number of the methylene chain of R1 of the radical shown by the general formula [1] concerning this invention from the data of a table 12, Although the holding time will decrease from the comparison of the data at the time of using the denaturation silica gel of the examples 12 and 13 of a table 12 as a bulking agent if a trimethylsilyl radical is changed into a dimethylsilyl radical, as for separation of a drug, it also turns out that it is good.

[0146] Example 26 The stainless steel column for HPLC with a bore [of 4.6mm] and a length of 150mm was filled up with the phenobarbital by direct impregnation of a blood serum sample, carbamazepine, and the silica gel manufactured in the assay example 12 of phenytoin with slurry method. It is phenobarbital to fetal calf serum and fetal calf serum at the analysis conditions shown below using this column. 20microg [ml] /, carbamazepine 5microg [ml] /and phenytoin What added ml in 40microg /was analyzed as a sample.

[0147] [Analysis conditions]

mobile phase: -- 100mM phosphate buffer solution (pH=6.9) / acetonitrile =90/10 (v/v) rate-of-flow: -- 1.2 ml/min measurement temperature: -- 30-degree-C detection wavelength: -- 254nm sample injection rate: -- 20microl [0148] The obtained chromatogram is shown in drawing 1 and drawing -2. Drawing 1 is what made fetal calf serum the sample, and it turns out that it is eluted without appearing immediately after the peak of fetal-calf-serum protein pouring in, i.e., fetal-calf-serum protein, being adsorbed by the column. Drawing -2 is what made the sample what added the three above-mentioned sorts (phenobarbital, carbamazepine, phenytoin) of drugs to fetal calf serum, and it turns out that each peak of phenobarbital, carbamazepine, and phenytoin is eluted, and it dissociates good after the peak of fetal-calf-serum protein. In addition, among drawing 2 , in one, 2 shows the peak of carbamazepine and 3 shows the peak of phenytoin for the peak of phenobarbital, respectively.

[0149] Example 27 It is lidocaine to fetal calf serum and fetal calf serum at the analysis conditions shown below using the column prepared in the assay example 26 of the lidocaine by direct impregnation of a blood serum sample. What added ml in 150microg /was analyzed as a sample.

[Analysis conditions]

mobile phase: -- 100mM phosphate buffer solution (pH=6.9) / acetonitrile =95/5 (v/v) rate-of-flow: -- 1.0 ml/min measurement temperature: -- 30-degree-C detection wavelength: -- 254nm sample injection rate: -- 20microl [0150] The obtained chromatogram is shown in Fig. -3 and -4. Drawing -3 is what made fetal calf serum the sample, and it turns out that it is eluted without appearing immediately after the peak of fetal-calf-serum protein pouring in, i.e., fetal-calf-serum protein, being adsorbed by the column. Drawing -4 is what made the sample what added lidocaine to fetal calf serum, and it turns out that the peak (** mark) of lidocaine is eluted and a blood serum component and fitness dissociate after the peak of fetal-calf-serum protein.

[0151] Example 28 It is primidone to fetal calf serum and fetal calf serum at the analysis conditions shown below using the column prepared in the assay example 26 of the primidone by direct impregnation of a blood serum sample. What added ml in 100microg /was analyzed as a sample.

[Analysis conditions]

mobile phase: -- 100mM phosphate buffer solution (pH=6.9) / acetonitrile =98/2 (v/v) rate-of-flow: -- 1.0 ml/min measurement temperature: -- 30-degree-C detection wavelength: -- 230nm sample injection rate: -- 20microl [0152] The obtained chromatogram is shown in Fig. -5 and -6. Drawing -5 is what made fetal calf serum the sample, and it turns out that it appears immediately after the peak of fetal-calf-serum protein pouring in. Drawing -6 is what made the sample what added primidone to fetal calf serum, and it turns out that the peak (** mark) of primidone is eluted and a blood serum component and fitness dissociate after the peak of fetal-

calf-serum protein.
[0153] Example 29 The stainless steel column for HPLC with a bore [of 4.6mm] and a length of 35mm was filled up with the phenobarbital in the blood serum sample by the column switching method, carbamazepine, and the silica gel manufactured in the assay example 12 of phenytoin with slurry method, and it considered as the column for pretreatment. The sample (they are phenobarbital 20microg/ml and carbamazepine to a man blood serum 5microg [ml] /and phenytoin what added ml in 40microg /) was analyzed by the column switching method using the equipment shown in drawing -7. That is, while condensing the drug in a sample with the column for pretreatment by passing the mobile phase for pretreatment to the passage shown by the dotted line of a bulb, serum protein was eliminated out of the column. Next, the bulb was changed and it analyzed for after [sample impregnation] 4 minutes to 5 minutes by introducing the drug in the column for pretreatment into the column for analysis by passing the mobile phase for analysis to the passage shown as the continuous line of a bulb. In addition, pretreatment conditions and analysis conditions are shown below.
[0154] [Pretreatment conditions]
mobile phase: -- 100mM phosphate buffer solution (pH=6.9) rate-of-flow: -- 1.0 ml/min measurement temperature: -- 30-degree-C sample injection rate: -- 20microl [0155] [Analysis conditions]
The column for analysis: Wakosil-II 5C18RS, 4.6phix250mm (product made from Wako Pure Chem Industry)
mobile phase: -- 100mM phosphate buffer solution (pH=6.9) / acetonitrile =65/35 (v/v) rate-of-flow: -- 1.0 ml/min measurement temperature: -- 30-degree-C detection wavelength: -- 254nm
[0156] The obtained chromatogram is shown in drawing -8. ** from drawing -8 shows like and that each peak of 1. phenobarbital, 2. carbamazepine, and 3. phenytoin dissociates good in order, appears, and can carry out the quantum of each drug good.
[0157] Example 30 The sample (it is imipramine hydrochloride to a man blood serum what added 200 ng/ml) was analyzed using the column for pretreatment prepared in the assay example 29 of the imipramine in the blood serum sample by the column switching method by the column switching method by the shown equipment which is shown in drawing -9. That is, while condensing the drug in a sample with the column for pretreatment by passing the mobile phase for pretreatment to the passage shown by the dotted line of a bulb, serum protein was eliminated out of the column. Next, the bulb was changed and the drug in the column for pretreatment was introduced into the column for analysis for after [sample impregnation] 6 minutes to 11 minutes by passing the mobile phase for pretreatment to the passage shown as the continuous line of a bulb. It analyzed by changing a bulb and returning the mobile phase for analysis to the passage which shows the dotted line of a bulb again. In addition, pretreatment conditions and analysis conditions are shown below.
[0158] [Pretreatment conditions]
mobile phase: -- 100mM phosphate buffer solution (pH=6.9) / acetonitrile =98/2 (v/v) rate-of-flow: -- 1.0 ml/min measurement temperature: -- 30-degree-C sample injection rate: -- 100microl [0159] [Analysis conditions]
The column for analysis: Wakosil-II 5C18RS, 4.6phix250mm (Wako Pure Chem Industry)
mobile phase: -- 100mM phosphate buffer solution (pH=2.5) / acetonitrile =65/35 (v/v) rate-of-flow: -- 1.0 ml/min measurement temperature: -- 30-degree-C detection wavelength: -- 254nm
[0160] The obtained chromatogram is shown in drawing 10 (the peak of imipramine: ** mark). Since the minor constituent in a blood serum can be easily condensed by using the column which comes to contain the denaturation silica gel of this invention as a bulking agent as a pretreatment column of a blood serum sample from this result, analysis of the minor constituent in a blood serum understands that the column concerning this invention is useful.
[0161] Example 31 The sample (it is indomethacin to a man blood serum what added ml in 1microg /) was analyzed using the column for pretreatment prepared in the assay example 29 of the indomethacin in the blood serum sample by the column switching method by the column switching method by the equipment shown in drawing -9. That is, while condensing the drug in a sample with the column for pretreatment by passing the mobile phase for pretreatment to the

passage shown by the dotted line of a bulb, serum protein was eliminated out of the column. Next, the bulb was changed and the sample in the column for pretreatment was introduced into the column for analysis for after [sample impregnation] 4.5 minutes to 8.5 minutes by passing the mobile phase for pretreatment to the passage shown as the continuous line of a bulb. It analyzed by returning to the passage which changes a bulb again and shows the mobile phase for analysis by the dotted line of a bulb. In addition, pretreatment conditions and analysis conditions were shown below.

[0162] [Pretreatment conditions]

mobile phase: -- 100mM phosphate buffer solution (pH=6.9) / acetonitrile =87/13 (v/v) rate-of-flow: -- 1.0 ml/min measurement temperature: -- 30-degree-C sample injection rate: -- 20microl [0163] [Analysis conditions]

column for analysis: -- Wakosil-II 5C18RS and 4.6phix250mm mobile phase:100mM phosphate buffer solution (pH=2.5) / acetonitrile =45/55 (v/v) rate-of-flow: -- 1.0 ml/min measurement temperature: -- 30-degree-C detection wavelength: -- 254nm [0164] The obtained chromatogram is shown in drawing 11 (the peak of indomethacin: ** mark). Since the minor constituent in a blood serum can be easily condensed by using the column which comes to contain the denaturation silica gel of this invention as a bulking agent as a pretreatment column of a blood serum sample from this result, analysis of the minor constituent in a blood serum understands that the column concerning this invention is useful.

[0165]

[Effect of the Invention] Denaturation silica gel with this invention useful as a bulking agent for chromatographies for analyzing the drug and metabolite in the biological material which contains protein components, such as a blood serum, so much as stated above, It is what offers the column for chromatographies which comes to fill [this bulking agent] up the analytical method list of the component in the biological material using the bulking agent for chromatographies and this which come to contain this denaturation silica gel. If biological materials, such as a blood serum, are analyzed using the column filled up with the denaturation silica gel of this invention as a bulking agent Macromolecules, such as protein, are bypassed without denaturalizing and are discharged out of a column, and on the other hand, comparatively low-molecular compounds, such as a drug in a sample, are condensed, and since separation elution is carried out, they do so the effect that the drug in a biological material etc. can be analyzed, without performing deproteination actuation. Moreover, using the above-mentioned **** property, the column filled up with the denaturation silica gel of this invention as a bulking agent is useful also as a pretreatment column for deproteination of a sample, and does so the effect of making it possible to analyze the low-concentration drug in biological materials, such as a blood serum, etc. to high sensitivity, by combining this and a common opposition system column.

[0166] Furthermore, since the denaturation silica gel of this invention is what is obtained by introducing the same qualification radical into the internal surface and outside surface of silica gel, it can obtain the bulking agent of quality with the easily same manufacture with sufficient repeatability. Furthermore, the denaturation silica gel of this invention also does so the effect that the holding time of the target analysis object can be adjusted suitably again, by adjusting the length of the methylene chain of the qualification radical to introduce.

[0167]

[Translation done.]

* NOTICES *

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Drawing 1 shows the chromatogram at the time of making fetal calf serum into a sample obtained in the example 26.
[Drawing 2] Drawing 2 shows the chromatogram at the time of making into a sample what added various drugs to the fetal calf serum obtained in the example 26.
[Drawing 3] Drawing 3 shows the chromatogram at the time of making into a sample the fetal calf serum obtained in the example 27.
[Drawing 4] Drawing 4 shows chromatogram, when what added lidocaine to the fetal calf serum obtained in the example 27 is made into a sample.
[Drawing 5] Drawing 5 shows the chromatogram at the time of making into a sample the fetal calf serum obtained in the example 28.
[Drawing 6] Drawing 6 shows chromatogram, when what added primidone to the fetal calf serum obtained in the example 28 is made into a sample.
[Drawing 7] Drawing 7 shows the equipment used by the column switching method of an example 29.
[Drawing 8] Drawing 8 shows the chromatogram obtained in the example 29.
[Drawing 9] Drawing 9 shows the equipment used by the column switching method of an example 30 and an example 31.
[Drawing 10] Drawing 10 shows the chromatogram obtained in the example 30.
[Drawing 11] Drawing 11 shows the chromatogram obtained in the example 31.
[0168]
[Description of Notations]
1 ... The peak of phenobarbital, 2 ... The peak of carbamazepine, 3 ... Peak of phenytoin.

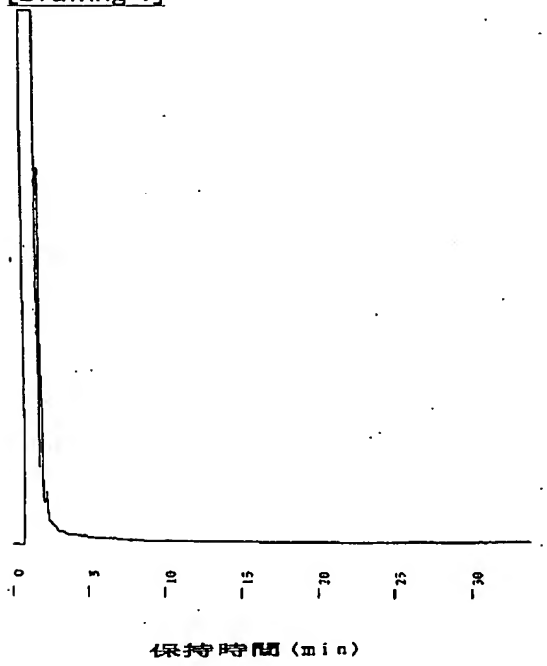
[Translation done.]

* NOTICES *

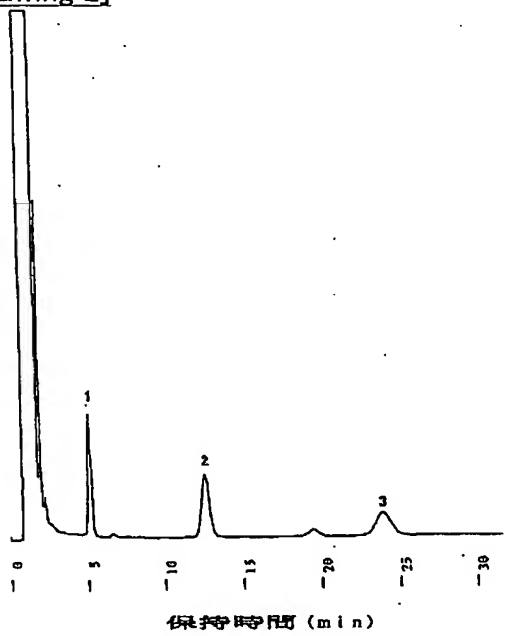
1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DRAWINGS

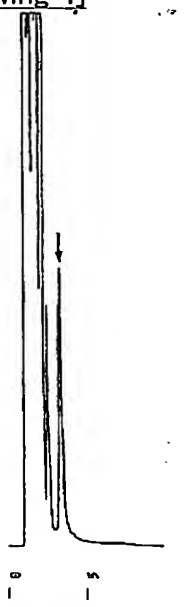
[Drawing 1]



[Drawing 2]

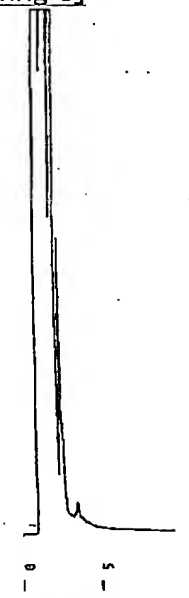


[Drawing 4]



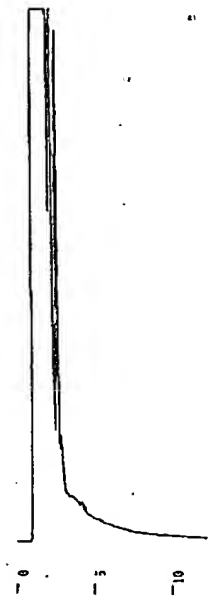
保持時間（min）

[Drawing 3]

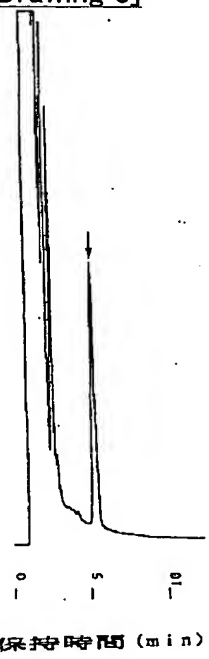


保持時間（min）

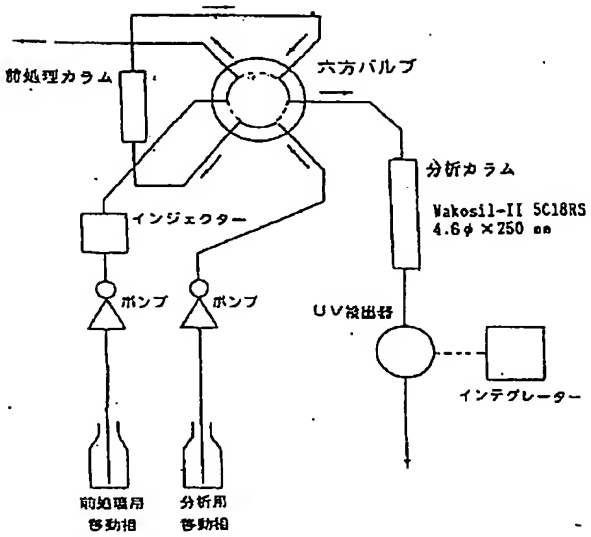
[Drawing 5]



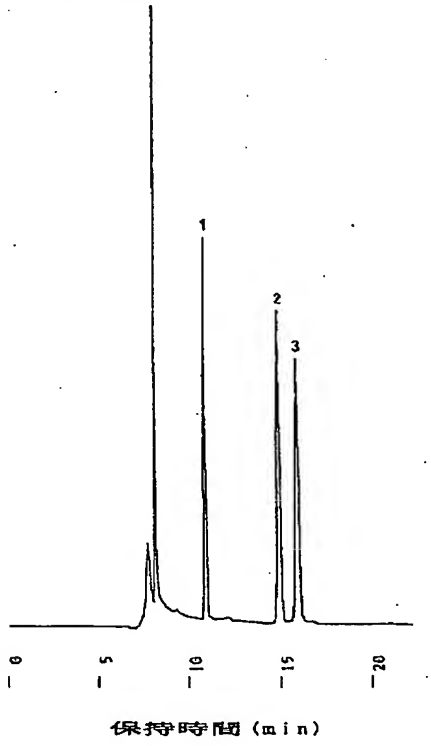
[Drawing 6]



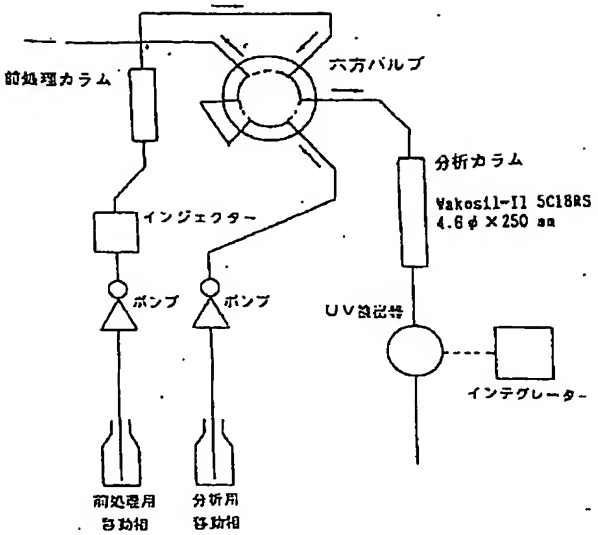
[Drawing 7]



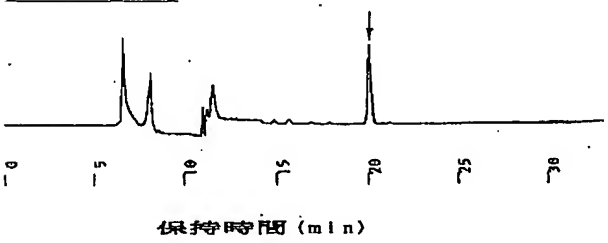
[Drawing 8]



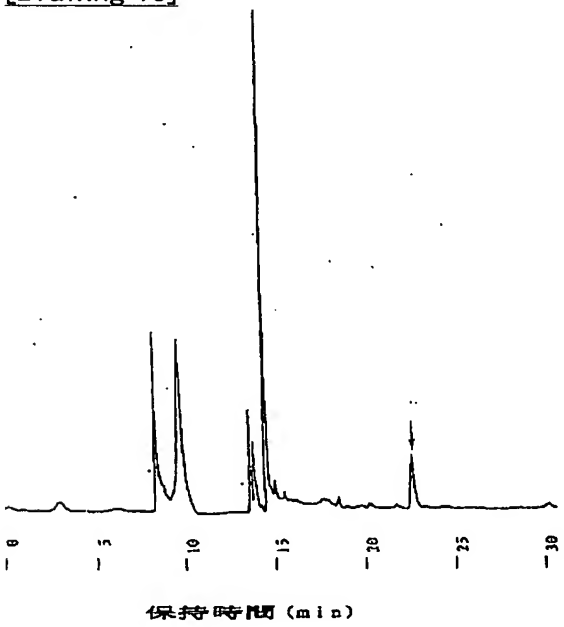
[Drawing 9]



[Drawing 11]



[Drawing 10]



[Translation done.]